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From: Chen, Shin-Lin
Sent: Wednesday, July 10, 2002 1:56 PM
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Subject: Publication

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Serial NO. 09/457,931.

WO 97/15690 A, May 1, 1997.. 34808

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STIC-ILL

10 7/10

From: Chen, Shin-Lin
Sent: Wednesday, July 10, 2002 3:38 PM
To: STIC-ILL
Subject: articles

403366

Please provide the following articles. thanks!
Serial NO. 09/457,931.

L18 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2002 ACS
AU Craig, J. C.; Westerman, M. E.; Bennett, G. D.; DiMichele, L.; Finnell, R. H.
TI Screening for reproductive toxicity in Fundulus heteroclitus by genetic expression profiling
SO Biomarkers (1996), 1(2), 123-135

L18 ANSWER 21 OF 24 MEDLINE
AU Mackler S A; Bennett G D; Tsuei V P; Finnell R H
TI Cocaine selectively alters neurotransmitter receptor mRNAs in mouse embryos.
SO REPRODUCTIVE TOXICOLOGY, (1996 Jan-Feb) 10 (1) 37-42.

L18 ANSWER 18 OF 24 MEDLINE
AU Wlodraczyk B; Bennett G D; Calvin J A; Craig J C; Finnell R H
TI Arsenic-induced alterations in embryonic transcription factor gene expression: implications for abnormal neural development.
SO DEVELOPMENTAL GENETICS, (1996) 18 (4) 306-15.

L18 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AU Morishima, M.; Yasui, H.; Nakazawa, M.
TI Expression pattern of nodal gene in the mouse embryo with visceroatrial heterotaxy induced by retinoic acid.
SO Congenital Anomalies, (Sept., 1997) Vol. 37, No. 3, pp. 315.
Meeting Info.: 37th Annual Meeting of the Japanese Teratology Society Kyoto, Japan July 14-16, 1997 Japanese Teratology Society

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No 7/10

From: Chen, Shin-Lin
Sent: Wednesday, July 10, 2002 3:38 PM
To: STIC-ILL
Subj ct: articles

4033677

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Serial NO. 09/457,931.

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L18 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2002 ACS
AU Craig, J. C.; Westerman, M. E.; Bennett, G. D.; DiMichele, L.; Finnell, R. H.
TI Screening for reproductive toxicity in Fundulus heteroclitus by genetic expression profiling
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09/457,931:047

From: Chen, Shin-Lin
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T : STIC-ILL
Subject: articles

Please provide the following articles. thanks!
Serial NO. 09/457,931.

L18 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2002 ACS
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L18 ANSWER 18 OF 24 MEDLINE
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L18 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
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STIC-ILL RSI, 368

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Sent: Wednesday, July 10, 2002 1:54 PM
T : STIC-ILL
Subject: article

Please provide the following article. Thanks!
Serial No. 09/457,931.

Spielmann et al., In Vitro Toxicity, 10: 119-127, 1997.

Maier et al., "Automated Array Technologies for Gene Expression Profiling", Drug Discov ry Today, GB, Elseview Science Ltd, Vol. 2, No. 8, p. 315-324, August, 1997.

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From: Chen, Shin-Lin
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Subject: articles

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Serial NO. 09/457,931.

L18 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2002 ACS
AU Craig, J. C.; Westerman, M. E.; Bennett, G. D.; DiMichele, L.; Finnell, R. H.
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STIC-ILL 40364

From: Chen, Shin-Lin
Sent: Wednesday, July 10, 2002 1:54 PM
To: STIC-ILL
Subject: article

Please provide the following article. Thanks!
Serial No. 09/457,931.

? Toxicology

Spielmann et al., In Vitro Toxicity, 10: 119-127, 1997. 0886-3954

Maier et al., "Automated Array Technologies for Gene Expression Profiling", Drug Discovery Today, GB, Elseview Science Ltd, Vol. 2, No. 8, p. 315-324, August, 1997.

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     (FILE 'HOME' ENTERED AT 15:13:35 ON 10 JUL 2002)

     FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 15:13:44 ON 10 JUL
     2002
L1      9721491 S EMBRYO OR FETUS OR ANIMAL
L2         6213 S MOLECULAR(5A)PROFILE
L3        41888 S (GENE OR PROTEIN)(8A)(EXPRESSION(5A)PATTERN)
L4        48015 S L2 OR L3
L5        25463 S L1 AND L4
L6        32815 S (GENE OR PROTEIN)(4A)(EXPRESSION(5A)PATTERN)
L7         1179 S L1 AND L2
L8       706484 S EMBRYO OR FETUS
L9           98 S L8 AND L2
L10       38948 S L6 OR L2
L11        6949 S L10 AND L8
L12        7695 S (GENE OR PROTEIN)(W)(EXPRESSION(5A)PATTERN)
L13      914542 S TOXICITY
L14          38 S L11 AND L13
L15          24 DUP REM L14 (14 DUPLICATES REMOVED)
L16         479 S L4 AND L13
L17         275 S L1 AND L10 AND L13
L18          24 DUP REM L15 (0 DUPLICATES REMOVED)

=> d au ti so ab 1-24 l18

L18  ANSWER 1 OF 24  CAPLUS  COPYRIGHT 2002 ACS
AU   Borrell, Antonio; Cruz Cutanda, M.; Lumbreras, Victoria; Pujal, Judit;
     Goday, Adela; Culianez-Macia, Francisco A.; Pages, Montserrat
TI   Arabidopsis thaliana Atrab28: a nuclear targeted protein related to
     germination and toxic cation tolerance
SO   Plant Molecular Biology (2002), 50(2), 249-259
     CODEN: PMBIDB; ISSN: 0167-4412
AB   The Arabidopsis gene Atrab28 has been shown to be expressed during late
     embryogenesis.  The pattern of expression of Atrab28
     mRNA and protein during embryo development is largely
     restricted to provascular tissues of mature embryos, and in
     contrast to the maize Rab28 homolog it cannot be induced by ABA and
     dehydration in vegetative tissues.  Here, we have studied the subcellular
     location of Atrab28 protein and the effect of its over-expression in
     transgenic Arabidopsis plants.  The Atrab28 protein was mainly detected
in
     the nucleus and nucleolus of cells from mature embryos.  In
     frame fusion of Atrab28 to the reporter green fluorescent protein (GFP)
     directed the GFP to the nucleus in transgenic Arabidopsis and in
     transiently transformed onion cells.  Anal. of chimeric constructs
     identified an N-terminal region of 60 amino acids contg. a five amino
acid
     motif QPKRP that was necessary for targeting GFP to the nucleus.  These
     results indicate that Atrab28 protein is targeted to the nuclear
     compartments by a new nuclear localization signal (NLS).  Transgenic
     Arabidopsis plants, with gain of Atrab28 function, showed faster
     germination rates under either std. or salt and osmotic stress
conditions.
     Moreover, improved cation toxicity tolerance was also obsd. not
     only during germination but also in seedlings.  These results suggest a
     role of Atrab28 in the ion cell balance during late embryogenesis and
     germination.
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L18 ANSWER 2 OF 24 MEDLINE
AU Rosen Mitchell B; Chernoff Neil
TI 5-Aza-2'-deoxycytidine-induced cytotoxicity and limb reduction defects in the mouse.
SO TERATOLOGY, (2002 Apr) 65 (4) 180-90.
Journal code: 0153257. ISSN: 0040-3709.
AB BACKGROUND: 5-Aza-2'-deoxycytidine (dAZA), causes hindlimb phocomelia in CD-1 mice. Studies in our laboratory have examined the hypothesis that compound- induced changes in **gene expression** may uniquely affect hindlimb **pattern** formation. The present study tests the hypothesis that dAZA causes limb dysplasia by inducing cytotoxicity among rapidly proliferating cells in the limb bud mesenchyme.
METHODS: Pregnant CD-1 mice were given a teratogenic dose of dAZA (i.p.) at different times on GD 10 and **fetuses** evaluated for skeletal development in both sets of limbs by standard methods. Using general histology and BrdU immunohistochemistry, limb mesenchymal cell death and cell proliferation were then assessed in **embryos** at various times post dosing, shortly after initial limb bud outgrowth. The effect of dAZA on early limb chondrogenesis was also studied using Northern analysis of scleraxis and Alcian blue staining of whole mount limb buds. RESULTS: Compound related hindlimb defects were not restricted to a specific set of skeletal elements but consisted of a range of temporally related limb anomalies. Modest defects of the radius were observed as well. These results are consistent with a general insult to the limb mesenchyme. Mesenchymal cell death and reduced cell proliferation were also observed in both sets of limbs. The timing and location of these effects indicate a role for cytotoxicity in the etiology of dAZA induced limb defects. These effects also agree with the greater teratogenicity of dAZA in the hindlimb because they were more pronounced in that limb. The expression of scleraxis, a marker of early chondrogenesis, was reduced 12 hr after dAZA exposure, a time coincident with maximal cell death, as was the subsequent emergence of Alcian blue stained long bone anlagen. CONCLUSIONS: These findings support the hypothesis that cytotoxic changes in the limb bud mesenchyme during early limb outgrowth can induce the proximal limb truncations characteristic of phocomelia after dAZA administration.

L18 ANSWER 3 OF 24 CAPLUS
IN Wu, J. H. David; Mantalaris, Athanassios
TI Cell culture system for culture of stromal and hemopoietic stem cells to make immune cells and uses including as human ex vivo immune system
SO PCT Int. Appl., 75 pp.
CODEN: PIXXD2
AB The present invention provides cultured immune system cells and methods of producing same. The method comprises culturing stromal cells and hemopoietic stem cells in a chamber having a scaffolding covered or surrounded with culture medium, wherein the scaffolding allows for hemopoietic stem cells and stromal cells to have cell to cell contacts in three dimensions. The subject immune system cells are useful for screening drugs which inhibit or stimulate the immune system. The subject

immune system cells are also useful in treating diseases of the immune system.

L18 ANSWER 4 OF 24 MEDLINE
AU Rohwedel J; Guan K; Hegert C; Wobus A M
TI Embryonic stem cells as an in vitro model for mutagenicity, cytotoxicity and embryotoxicity studies: present state and future prospects.
SO TOXICOLOGY IN VITRO, (2001 Dec) 15 (6) 741-53. Ref: 122
Journal code: 8712158. ISSN: 0887-2333.
AB Primary cultures or established cell lines of vertebrates are commonly used to analyse the mutagenic, embryotoxic or teratogenic potential of environmental factors, drugs and xenobiotics in vitro. However, these cellular systems do not include developmental processes from early embryonic stages up to terminally differentiated cell types. An alternative approach has been offered by permanent lines of pluripotent stem cells of embryonic origin, such as embryonic carcinoma (EC), embryonic stem (ES) and embryonic germ (EG) cells. The undifferentiated stem cell lines are characterized by nearly unlimited self-renewal capacity and have been shown to differentiate in vitro into cells of all three primary germ layers. Pluripotent embryonic stem cell lines recapitulate cellular developmental processes and **gene expression patterns** of early embryogenesis during in vitro differentiation, data which are summarized in this review. In addition, recent studies are presented which investigated mutagenic, cytotoxic and embryotoxic effects of chemical substances using in vitro systems of pluripotent embryonic stem cells. Furthermore, an outlook is given on future molecular technologies using embryonic stem cells in developmental toxicology and embryotoxicology.

L18 ANSWER 5 OF 24 CAPLUS COPYRIGHT 2002 ACS
AU Vinson, Robert K.; Hales, Barbara F.
TI Expression of base excision, mismatch, and recombination repair genes in the organogenesis-stage rat conceptus and effects of exposure to a genotoxic teratogen, 4-hydroperoxycyclophosphamide
SO Teratology (2001), 64(6), 283-291
CODEN: TJADAB; ISSN: 0040-3709
AB DNA repair capability may influence the outcome of genotoxic teratogen exposure. The goals of this study were to assess the expression of base excision repair (BER), mismatch repair (MMR), and recombination repair (RCR) genes in the mid-organogenesis rat conceptus and to det. the effects on expression of exposure to the genotoxic teratogen, 4-hydroperoxycyclophosphamide (4-OOHCPA). The expression of 17 BER, MMR, and RCR genes was examd. in gestational day (GD) 10-12 rat conceptuses using the antisense RNA (aRNA) technique. **Embryos** were cultured with 10 .mu.M 4-OOHCPA to examine effects on gene expression. Yolk sacs and **embryos** had similar **gene expression patterns** for all three DNA repair pathways from GD10-12. Transcripts for APNG, PMS1, and RAD54 were present at high concns. in both tissues. The remainder of the genes were expressed at low levels in yolk sac, with a few not detected on GD10 and 11. In the **embryo**, transcripts for most genes were low on GD10 and 11; several increased by GD12. After exposure to 4-OOHCPA for 24 h, XRCC1 and RAD57 expression decreased in yolk sac, whereas only RAD51 transcripts decreased in the **embryo**. By 44 h, transcripts for all BER genes decreased in yolk sac; in the **embryo**, most BER, MMR, and RCR genes decreased, many below the level of detection. The expression of DNA repair genes in the mid-organogenesis rat conceptus is varied and subject to down-regulation by 4-OOHCPA. DNA repair gene expression may det. the consequences of

genotoxicant exposure during development.

L18 ANSWER 6 OF 24 MEDLINE
AU Nasrallah I; Golden J A
TI Brain, eye, and face defects as a result of ectopic localization of Sonic hedgehog protein in the developing rostral neural tube.
SO TERATOLOGY, (2001 Aug) 64 (2) 107-13.
Journal code: 0153257. ISSN: 0040-3709.
AB BACKGROUND: Normal development of the face, eyes, and brain requires the coordinated expression of many genes. One gene that has been implicated in the development of each of these structures encodes the secreted protein, Sonic hedgehog (Shh). During central nervous system development, Shh is required for ventral specification along the entire neural axis. To further explore the role of Shh in chick brain and craniofacial development, we overexpressed Shh in the developing rostral neural tube METHODS: In order to determine if Shh is sufficient to ventralize the forebrain, we localized ectopically recombinant Shh protein to the rostral neural tube of chick **embryos**. The resulting **embryos** were evaluated morphologically and by assaying gene expression. RESULTS: Disruption in normal **gene expression patterns** was observed with a reduction or loss in expression of genes normally expressed in the dorsal forebrain (wnt-3a, wnt-4, and Pax-6) and expansion of ventrally expressed genes dorsally (HNF-3beta, Ptc). In addition to the genetic alterations observed in the neural tube, a craniofacial phenotype characterized by a reduction in many cranial neural crest-derived structures was observed. The eyes of Shh-treated **embryos** were also malformed. They were small with expansion of the retinal pigmented epithelium, enlarged optic stalks, and a reduction of neural retina. DISCUSSION: The ectopic localization of recombinant Shh protein in the rostral neural tube resulted in severe craniofacial anomalies and alterations of gene expression predicted by other studies. The system employed appears to be a model for studying the embryogenesis of malformations that involve the brain, eyes, and face.

L18 ANSWER 7 OF 24 CAPLUS
IN Snodgrass, H. Ralph
TI **Toxicity** typing using embryoid bodies
SO PCT Int. Appl., 56 pp.
CODEN: PIXXD2
AB This invention provides methods and systems for identifying and typing **toxicity** of chem. compns., as well as for screening new compns. for **toxicity**. The invention involves detecting alterations in gene or protein expression and hence establishing **mol. profiles** in isolated mammalian embryoid bodies contacted with various chem. compns. of known and unknown **toxicities**, and correlating the **mol. profiles** with **toxicities** of the chem. compns.

L18 ANSWER 8 OF 24 BIOSIS
AU Block, Karen; Kardana, Andrew; Igarashi, Peter; Taylor, Hugh S. (1)
TI In utero diethylstilbestrol (DES) exposure alters Hox gene expression in the developing mullerian system.
SO FASEB Journal, (June, 2000) Vol. 14, No. 9, pp. 1101-1108. print.
ISSN: 0892-6638.
AB Diethylstilbestrol (DES) was widely used to treat pregnant women through

1971. The reproductive tracts of their female offspring exposed to DES in utero are characterized by anatomic abnormalities. Here we show that DES administered to mice in utero produces changes in the **expression pattern** of several Hox **genes** that are involved in patterning of the reproductive tract. DES produces posterior shifts in Hox gene expression and homeotic anterior transformations of the reproductive tract. In human uterine or cervical cell cultures, DES induces HOXA9 or HOXA10 gene expression, respectively, to levels approximately twofold that induced by estradiol. The DES-induced expression is not inhibited by cyclohexamide. Estrogens are novel morphogens that directly regulate the **expression pattern** of posterior Hox **genes** in a manner analogous to retinoic acid regulation of anterior Hox genes. Alterations in HOX gene expression are a molecular mechanism by which DES affects reproductive tract development. Changes in Hox gene expression are a potential marker for the effects of in utero drug use that may become apparent only at late stages of development.

L18 ANSWER 9 OF 24 MEDLINE
AU Finnell R H; Gelineau-van Waes J; Bennett G D; Barber R C; Wlodarczyk B; Shaw G M; Lammer E J; Piedrahita J A; Eberwine J H
TI Genetic basis of susceptibility to environmentally induced neural tube defects.
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (2000) 919 261-77.
Journal code: 7506858. ISSN: 0077-8923.
AB Neural tube defects (NTDs) are among the most common of all human congenital defects, with multifactorial etiologies comprising both environmental and genetic components. Several murine model systems have been developed in an effort to elucidate genetic factors regulating expression of NTDs. Strain-dependent differences in susceptibility to teratogenic insults and altered **patterns** of **gene expression** observed within the neuroepithelium of affected **embryos** support the hypothesis that subtle genetic changes can result in NTDs. Since several affected genes are folate-regulated, transgenic knockout mice lacking a functional folate receptor were developed. Nullizygous **embryos** died in utero with significant morphological defects, supporting the critical role of folic acid in early embryogenesis. While epidemiological studies have not established an association between polymorphisms in the human folate receptor gene and NTDs, it is known that folate supplementation reduces infant NTD risk. Continued efforts are therefore necessary to reveal the mechanism by which folate works and the nature of the gene(s) responsible for human NTDs.

L18 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2002 ACS
AU Spielmann, Horst; Scholz, Gabriele; Pohl, Ingeborg; Genschow, Elke; Klemm, Martina; Visan, Anke
TI The use of transgenic embryonic stem (ES) cells and molecular markers of differentiation for improving the embryonic stem cell test (EST)
SO Congenital Anomalies (2000), 40(Suppl.), S8-S18
CODEN: CGANE7; ISSN: 0914-3505
AB A review with 23 refs. Blastocyst-derived pluripotent embryonic stem (ES) cells of the mouse can be induced to differentiate in culture into a variety of cell types, including cardiac muscle cells. In the embryonic stem cell test (EST) the capacity of ES cells of the mouse cell line D3 to

differentiate into contracting myocard cells is used to assess the embryotoxic potential of drugs, and, in addn., the effects on the viability of ES cells and differentiated mouse fibroblasts (cell line 3T3) are compared. The following endpoints are used to classify the embryotoxic potential of chems. after 10 days of exposure: (i) the inhibition of differentiation of ES cells into cardiomyocytes (ID50) and the decrease of viability of (ii) 3T3 cells (IC503T3) and (iii) ES (IC50D3) cells in a MTT cytotoxicity test. Applying linear anal. of discriminance, a biostatistical prediction model (PM) was developed to assign test chems. to three classes of embryotoxicity. Results obtained recently in validation studies under blind conditions in several labs. will be presented. Attempts have been made to improve the EST by establishing mol. endpoints of differentiation in cultured ES cells. A genetically engineered mouse ES cell line expressing green fluorescent protein (GFP) under the control of cardiac .alpha.-actin has successfully been used by Hescheler and co-workers. We have studied the expression of tissue specific proteins in ES cell cultures in the presence of embryotoxic chems. by immune fluorescent antibody techniques, e. g. FACS anal. Other groups are focusing on endogenous gene expression in early development by RT-PCR methods or on the DNA microarray technique. Mol. endpoints will allow to improve the EST by measuring **gene expression patterns** in a small no. of ES cells.

L18 ANSWER 11 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AU Yang, J. (1); Zoeller, R. T.
TI Limited changes in **patterns** of **gene expression** in alcohol-treated rat fetal brain revealed by differential display and cDNA array.
SO Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-582.14. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000 Society for Neuroscience . ISSN: 0190-5295.
AB Children born with Fetal Alcohol Syndrome show facial abnormalities, stunted growth and specific neurological deficits, including microcephaly.
In rats, prenatal alcohol exposure profoundly affects the proliferation and migration of cortical neurons (Miller, Science 233: 1308, 1986). To test whether global changes in the **pattern** of **gene expression** occur in the brain of the rat model, we used differential display and cDNA array technology. We evaluated changes of gene expression in gestational day (G) 13 and 16 brains, because these times represent the beginning and peak of cortical neurogenesis. Differential display was performed among ethanol, pairfed and control groups. 7 genes were identified on G13 with 24 unique primer pairs; 2 on G16 with 72 unique primer pairs. However, only Neuroendocrine Specific Protein-A (NSP-A) was confirmed to be up-regulated by ethanol in fetal cortex by in situ hybridization, while others could not be confirmed by northern and in situ hybridization. cDNA array was also performed between ethanol and pairfed groups on AtlasTM Rat 1.2 array, which has 1176 genes.
3 genes showed greater than 2-fold difference in expression on G13 and 2 on G16. These genes are not involved in the same specific biological process. The findings from both differential display and cDNA array suggest that the profound deleterious effects on brain development induced by alcohol may not be mediated by global changes in the **pattern** of **gene expression**.

L18 ANSWER 12 OF 24 MEDLINE
AU Torchinsky A; Ivnitsky I; Savion S; Shepshelovich J; Gorivodsky M; Fein A;
Carp H; Schwartz D; Frankel J; Rotter V; Toder V
TI Cellular events and the **pattern** of p53 **protein expression** following cyclophosphamide-initiated cell death in various organs of developing **embryo**.
SO TERATOGENESIS, CARCINOGENESIS, AND MUTAGENESIS, (1999) 19 (5) 353-67.
Journal code: 8100917. ISSN: 0270-3211.
AB This study was aimed at characterizing the temporal patterns of cell responses and p53 protein expression in the limbs, head, and liver of **embryos** responding to cyclophosphamide (CP)-induced teratogenic insult. ICR murine **embryos** were examined 24, 48, or 72 h after injection of 40 mg/kg CP on day 12 of pregnancy. The cellular events and temporal **pattern** of p53 **protein expression** were determined by FACS analysis and by TUNEL (apoptosis) in the head, limbs, and liver of the **embryos**. All tested organs showed apoptosis and a significantly decreased proportion of live cells after 24 h. Subsequent events were organ-dependent. In the liver, there were no dysmorphic events at any time and excessive cell death had been almost compensated for by 48 h. Compensation was preceded by G(1) arrest and accompanied by an increased level of p53 protein in surviving cells. Excessive cell death in the head and the limbs resulted in structural anomalies. In the head, there was an increased level of p53 protein and G(1) arrest after 24 h and the number of live cells at 48 h was equal to that seen in earlier samples, despite apoptosis. In the limbs, however, only isolated viable cells were seen by 48 h, but there was no increased level of p53 protein or G(1) arrest. Results of this study suggest that the differential sensitivity of tested organ systems to CP may be associated with differences in cellular events following CP-initiated cell death. They also suggest that the input of p53 in determining the response of these organ systems to CP-induced teratogenic insult may be different. Teratogenesis Carcinog. Mutagen. 19:353-367, 1999.

L18 ANSWER 13 OF 24 MEDLINE
AU Treinen K A; Louden C; Dennis M J; Wier P J
TI Developmental **toxicity** and toxicokinetics of two endothelin receptor antagonists in rats and rabbits.
SO TERATOLOGY, (1999 Jan) 59 (1) 51-9.
Journal code: 0153257. ISSN: 0040-3709.
AB **Embryo**-fetal development studies with toxicokinetic evaluations were conducted in rats and rabbits after oral or intravenous administration of two endothelin receptor antagonists. In the rat studies, females were administered SB-217242 (0.01-300 mg/kg/day) orally or SB-209670 (0.01-50 mg/kg/day) intravenously from days 6-17 postcoitus (pc). External and visceral fetal examinations were performed at necropsy on day 21 pc. Maternal body weight and food consumption were decreased only at 300 mg/kg/day SB-217242. Embryolethality was seen at 300 mg/kg/day SB-217242. Decreased fetal body weight occurred at 300 mg/kg/day SB-217242 and 50 mg/kg/day SB-209670. Dose-dependent increases in the mean percentage of **fetuses** per litter with malformations were seen at > or = 50 mg/kg/day SB-217242 and > or = 10 mg/kg/day SB-209670. Craniofacial, great vessel, heart, and thyroid were the predominant malformations. In the rabbit studies, females were administered SB-217242

(0.01-50 mg/kg/day) orally or SB-209670 (0.01-25 mg/kg/day) intravenously from days 6-20 pc. There was no drug-related effect on maternal body weight or food consumption. Embryolethality was observed at 50 mg/kg/day of SB-217242. Dose-related increases in the mean percentage of **fetuses** per litter with malformations were seen at > or = 10 mg/kg/day SB-217242 and > or = 10 mg/kg/day SB-209670. The malformations were similar to those observed in the rat studies, except that craniofacial development was not altered by SB-209670. The malformations observed are consistent with the **pattern** of endothelin-1 **gene expression** described in mouse embryonic pharyngeal arches and heart, and with the craniofacial and cardiovascular malformations observed in endothelin-1-deficient mice. Given the known role for endothelins in development, and concordant malformations in rats and rabbits observed in this study, teratogenicity is likely to be a class effect of endothelin receptor antagonists.

L18 ANSWER 14 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AU Clotman, F.; Van Maele-Fabry, G.; Picard, J. J. (1)
TI All-trans-retinoic acid upregulates the expression of COUP-TFI in early-somite mouse **embryos** cultured in vitro.
SO Neurotoxicology and Teratology, (Nov.-Dec., 1998) Vol. 20, No. 6, pp. 591-599.
ISSN: 0892-0362.
AB Exposure of **embryos** to an excess of retinoic acid (RA) modifies the spatio-temporal **pattern** of **expression** of developmental **genes**. RA regulates the expression of target genes through binding of the retinoid nuclear receptors (RARs and RXRs), as heterodimers, to regulatory cis-acting elements. COUP-TF factors, which are able to dimerize with the RXRs and to compete with the retinoid receptors for their DNA binding sites, are suspected to modulate the retinoid signal transduction pathway. Therefore, COUP-TF factors may be involved in the regulation of the expression of developmental genes and/or in the modifications induced by an excess of RA in the expression of these genes. The aim of this work is to assess whether RA-induced modifications in the expression of Krox-20 and Hox genes correlate with alterations of the expression of COUP-TF genes. In addition to spatial modifications in the expression patterns of Krox-20 and Hox genes, we report here an upregulation of the expression level of COUP-TFI after RA exposure. However, this abnormality did not spatially overlap with the modifications observed in the expression of Krox-20 and Hox genes. These data suggest an involvement of COUP-TFI in the generation of RA-induced abnormalities, but do not support the hypothesis of an involvement of this factor in the regulation of the expression of Hox or Krox-20 genes.

L18 ANSWER 15 OF 24 MEDLINE
AU Hartig P C; Hunter E S 3rd
TI Gene delivery to the neurulating **embryo** during culture.
SO TERATOLOGY, (1998 Sep-Oct) 58 (3-4) 103-12.
Journal code: 0153257. ISSN: 0040-3709.
AB Modulating expression of specific genes during embryogenesis will help elucidate their role in development. Transient overexpression of specific genes can be accomplished by adding additional copies, or else antisense transcripts can be used to block expression. Manipulation of gene expression requires an efficient, nontoxic gene delivery system. We

compared a plasmid and a replication-defective adenovirus (Ad5) as methods of delivering genes to the **embryo** during the neurulation stage of development. Both vectors utilized a construct containing the bacterial beta-galactosidase reporter gene under the control of the human cytomegalovirus early gene promoter and the SV40 polyadenylation signal. Vectors were delivered by intraamniotic microinjection to **embryos** prepared for whole-**embryo** culture. Plasmid transfection experiments were done with and without polycationic lipid (lipofectamine, 20 or 125 micrograms/microliter) enhancement at 0.1 and 0.01 microgram per **embryo**. Twenty-six hours after transfection with plasmid only, **embryos** appeared normal, but had very weak gene expression which was detected only after extended periods of staining. In contrast, adenovirus gene delivery was successful. While high concentrations of virus (6 x 10(8) particles/ microliter) elicited significant malformations, lower concentrations (1.5 x 10(8) particles/microliter) produced no malformations and intense gene expression. Time-course studies revealed staining at 6 hr postinjection, and intense staining at 26 hr. Staining appeared primarily in the neurectoderm and cells derived from the neurectoderm. This **pattern** of **gene expression** was confirmed using a green fluorescent protein-expressing adenovirus. Rapid induction of gene expression with no **toxicity** is critical to the utility of this technique within the whole-**embryo** culture system. Clearly, Ad5 transduction provides a more useful tool than plasmid vectors.

L18 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AU Morishima, M.; Yasui, H.; Nakazawa, M.
TI **Expression pattern** of nodal **gene** in the mouse **embryo** with visceroatrial heterotaxy induced by retinoic acid.
SO Congenital Anomalies, (Sept., 1997) Vol. 37, No. 3, pp. 315. Meeting Info.: 37th Annual Meeting of the Japanese Teratology Society Kyoto, Japan July 14-16, 1997 Japanese Teratology Society . ISSN: 0914-3505.

L18 ANSWER 17 OF 24 MEDLINE
AU Iulianella A; Lohnes D
TI Contribution of retinoic acid receptor gamma to retinoid-induced craniofacial and axial defects.
SO DEVELOPMENTAL DYNAMICS, (1997 May) 209 (1) 92-104. Journal code: 9201927. ISSN: 1058-8388.
AB Exogenous retinoic acid (RA) administered during mouse embryogenesis can alter the pattern of the axial skeleton during two developmental periods: an early window (7 to 8.5 days post-coitum; dpc) and a late window (9.5 to 11.5 dpc). Treatment during the early window results in vertebral homeotic transformations (predominantly posteriorizations) concomitant with rostral shifts in Hox gene expression, while treatment at the later window results in similar transformations without detectable alterations in Hox **gene expression patterns**. Mice null for retinoic acid receptor gamma (RAR gamma) exhibit axial defects, including

homeosis of several vertebrae, therefore establishing a role for this receptor in normal axial specification RAR gamma null mutants are also completely resistant to RA-induced spina bifida, which occurs in wildtype **embryos** treated at 8.5-9.0 dpc, suggesting that this receptor specifically transduces at least a subset of the teratogenic effects of retinoids. To further investigate the role of RAR gamma in RA-induced defects during the early and late windows of retinoid-sensitive vertebral patterning, RAR gamma heterozygotes were intercrossed, pregnant females treated with vehicle or RA at 7.3, 10.5 or 11.5 dpc and full-term **fetuses** assessed for skeletal defects. Relative to wildtype littermates, RAR gamma null mutants treated at 7.3 dpc were markedly resistant to RA-induced embryolethality, craniofacial malformations, and neural tube defects. Furthermore, while RAR gamma null mutants were modestly resistant to certain vertebral malformations elicited by RA treatment at 7.3, they exhibited more pronounced resistance following treatment at 10.5 and 11.5 dpc. Moreover, several of the vertebral defects inherent to the RAR gamma null phenotype were abolished by RA treatment specifically at 10.5 dpc, suggesting that RAR alpha and/or RAR beta isoforms may substitute for certain RAR gamma functions, and that RAR gamma may elicit its normal effects on vertebral morphogenesis at this developmental stage.

L18 ANSWER 18 OF 24 MEDLINE
AU Wlodraczyk B; Bennett G D; Calvin J A; Craig J C; Finnell R H
TI Arsenic-induced alterations in embryonic transcription factor gene expression: implications for abnormal neural development.
SO DEVELOPMENTAL GENETICS, (1996) 18 (4) 306-15.
Journal code: 7909963. ISSN: 0192-253X.
AB We examined the morphological and molecular consequences of acute in utero exposure to teratogenic concentrations of arsenate. The treatment produced a dose-related increase in neural tube defects, along with a significant alteration in the **pattern** of **gene expression** for several transcription factors (creb, Hox 3.1, Pax3, and Emx-1) that were examined using in situ transcription and antisense RNA amplification procedures. On gestational day 9:0, there was a significant delay in the **embryos** progression through neural tube closure, accompanied by a significant downregulation of Hox 3.1 expression and a significant upregulation of Pax3, Emx-1, and creb. As both Hox 3.1 and Pax3 serve to regulate N-CAM expression, it is possible that abnormalities associated with N-CAM may compromise neural crest cell migration and normal neural tube closure.

L18 ANSWER 19 OF 24 MEDLINE
AU Wlodarczyk B C; Craig J C; Bennett G D; Calvin J A; Finnell R H
TI Valproic acid-induced changes in gene expression during neurulation in a mouse model.
SO TERATOLOGY, (1996 Dec) 54 (6) 284-97.
Journal code: 0153257. ISSN: 0040-3709.
AB The teratogenic potential of valproic acid has been well established both in experimental models and in human clinical studies. As with all human teratogens, there are genetically determined differences in individual susceptibility to the induction of congenital defects. Using a mouse model of valproate-induced neural tube defects, a study was undertaken to examine differential changes in gene expression for selected transcription factor (Pax-3, Emx-1, Emx-2, c-fos, c-jun, creb) and cell cycle checkpoint

genes (bcl-2, p53, wee-1) during neural tube closure. In general, exposure to teratogenic concentrations of valproic acid elicited GD 9:12 control levels of transcription factor mRNA expression in GD 9:0 **embryos** of both strains. This accelerated developmental profile is marked by significant elevation of Emx-1, Emx-2, c-fos, c-jun, and creb expression. There was also a significant over expression of the cell cycle genes p53 and bcl-2 in the LM/Bc **embryos** in response to the teratogenic insult. Examination of the ratio of expression of these genes clearly favored bcl-2, which supports the hypothesis that altered neuroepithelial cell proliferation rates, rather than increased apoptosis, is the underlying mechanism by which valproic acid alters normal neural tube morphogenesis. An investigation into interactive effects of these genes on the **molecular profile** of GD 9:0 **embryos** further validated this observation. That is, the overall proliferative state among the control **embryos** was prematurely modified into a more differentiated state following teratogenic insult. These results suggest that alterations in the expression of multiple genes are most likely responsible for valproic acid-induced neural tube defects.

L18 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2002 ACS

AU Craig, J. C.; Westerman, M. E.; Bennett, G. D.; DiMichele, L.; Finnell, R. H.

TI Screening for reproductive **toxicity** in Fundulus heteroclitus by genetic expression profiling

SO Biomarkers (1996), 1(2), 123-135
CODEN: BIOMFA; ISSN: 1354-750X

AB Potentially teratogenic agents enter the environment at a rate that greatly exceeds current capabilities to effectively evaluate their reproductive **toxicities**. This is due, in part, to costly, labor-intensive methodologies involving mammalian embryonic screening assays that are currently in use worldwide. Therefore, we sought to develop a rapid, less expensive screening system with which to identify mol. biomarkers of teratogenicity using a non-mammalian system. **Embryos** of the topminnow, Fundulus heteroclitus, offer several advantages in terms of reproductive **toxicity** screening efficiency as compared with mammalian embryonic systems. These **embryos** are easily manipulated and develop normally at ambient temp. in air, water, or air-satd. mineral oils, making them readily adapted for field studies. In the present study, developing F. heteroclitus **embryos** were exposed to teratogenic concns. of sodium valproate (VPA) or arsenic acid (arsenate), and the frequency and types of induced malformations were evaluated. Using in situ transcription and antisense RNA (aRNA) amplification procedures (IST/aRNA), we attempted to correlate the teratogenic outcomes to specific alterations in the expression of a panel of developmentally regulated genes. Preliminary studies identified treatment concns. of arsenate and VPA that induced abnormal development in 95% of the surviving **embryos**. Among the F. heteroclitus **embryos**, the structural defects most commonly induced by these compds. were cardiac and neural tube malformations. The genetic expression profiles revealed a no. of genes whose expression levels were significantly altered by exposure to the test compds. Mol. anal. of F. heteroclitus embryonic development represents a novel, inexpensive approach to screen for potential

teratogens, and identify **genes** whose **expression patterns** may be used as biomarkers, or indicators, of teratogenicity.

L18 ANSWER 21 OF 24 MEDLINE
AU Mackler S A; Bennett G D; Tsuei V P; Finnell R H
TI Cocaine selectively alters neurotransmitter receptor mRNAs in mouse **embryos**.
SO REPRODUCTIVE TOXICOLOGY, (1996 Jan-Feb) 10 (1) 37-42.
Journal code: 8803591. ISSN: 0890-6238.
AB Alterations in gene expression due to in utero cocaine exposure may adversely affect nervous system development. The present study examined whether or not cocaine administration to pregnant mice alters embryonic mRNA levels for several developmentally-regulated genes. Antisense RNA amplification was performed using RNA from LM/Bc **embryos** at gestational days 9.5 and 10.5 after three days of cocaine treatment. This technique highlights simultaneous changes that occur in the expression of many genes after a teratogenic insult. Significant changes occurred in the **expression pattern** on only four **genes** from a total of 42 candidate cDNAs. These included increases in the relative levels of the alpha and beta 1 subunits of the GABAA receptor without concurrent changes in the non-NMDA glutamate receptor subunits. The results support the hypothesis that in utero cocaine exposure leads to specific changes in gene expression that may ultimately contribute to developmental abnormalities.

L18 ANSWER 22 OF 24 MEDLINE
AU Abbott B D; Probst M R
TI Developmental expression of two members of a new class of transcription factors: II. Expression of aryl hydrocarbon receptor nuclear translocator in the C57BL/6N mouse **embryo**.
SO DEVELOPMENTAL DYNAMICS, (1995 Oct) 204 (2) 144-55.
Journal code: 9201927. ISSN: 1058-8388.
AB The aryl hydrocarbon receptor (AhR) and the AhR nuclear translocator protein (ARNT) are basic-helix-loop-helix (bHLH) proteins involved in transcriptional regulation. The AhR is a ligand-activated partner of the ARNT protein. Both proteins are required to transcriptionally regulate gene expression. ARNT must be complexed to AhR to permit binding to the regulatory DNA sequence. The AhR-ligand complex is known to mediate a range of biological responses, such as developmental **toxicity**, induction of cleft palate, and hydronephrosis. AhR and ARNT are expressed in human embryonic palatal cells and AhR was recently shown to have a specific developmental pattern of expression in the mouse **embryo**. In the present study, expression of ARNT is characterized in C57Bl/6N mouse **embryos** from gestation day (GD) 10-16 using immunohistochemistry and in situ hybridization. Af affinity purified antibody against human ARNT (1.1 micrograms/ml) was detected with an avidin-biotin-peroxidase complex. ARNT mRNA was localized with a 35S-RNA probe from pBM5/NEO-M1-1. Specific spatial and temporal **patterns** of ARNT **expression** emerged and mRNA and **protein** expression correlated. The GD 10-11 **embryos** showed highest levels of ARNT in neuroepithelial cells of the neural tube, visceral arches, otic and optic placodes, and preganglionic complexes. The heart also had significant expression of ARNT with strong nuclear localization. After GD11, expression in heart and brain declined. In GD 12-13 **embryos** expression was highest in the liver where expression increased from GD 12-16. At GD 15-16 the highest levels of ARNT occurred in adrenal gland and liver, although ARNT was also detected in submandibular gland, ectoderm, tongue, bone, and muscle. In all of these

tissues ARNT was cytoplasmic as well as nuclear, except in some of the cortical adrenal cells in which ARNT was strongly cytoplasmic with little or no nuclear localization. These specific patterns of ARNT expression, which differ in certain tissues from the expression of AhR, suggest that ARNT may have additional roles in normal embryonic development.

L18 ANSWER 23 OF 24 MEDLINE
AU Musselman A C; Bennett G D; Greer K A; Eberwine J H; Finnell R H
TI Preliminary evidence of phenytoin-induced alterations in embryonic gene expression in a mouse model.
SO REPRODUCTIVE TOXICOLOGY, (1994 Sep-Oct) 8 (5) 383-95.
Journal code: 8803591. ISSN: 0890-6238.
AB SWV mouse **embryos** collected on gestational days (GD) 9:12 and 10:00 following chronic in utero exposure to teratogenic concentrations of phenytoin were utilized for in situ transcription studies of gene expression. The substrate cDNA obtained from the frozen **embryo** sections was amplified into radiolabelled antisense RNA (RT/aRNA) and used as a probe to screen a panel of 20 cDNA clones representing genes that are important regulators of craniofacial and neural development. The magnitude of alteration in gene expression following phenytoin treatment was determined densitometrically by changes in the hybridization intensity of the aRNA probes to the cDNA clones immobilized to the slot blots. We found that both Wnt-1 and the calcium channel gene were developmentally regulated, as their level of expression decreased significantly between the two collection times. Phenytoin treatment produced a significant downregulation in the level of expression for 25% of the genes examined in the GD 9:12 **embryos**, including the growth factors TGF-beta and NT3, the proto-oncogene Wnt-1, the nicotinic receptor, and the voltage sensitive calcium channel gene. Additional changes in the coordinate expression of several of the growth and transcription factors were observed at both gestational timepoints. The application of RT/aRNA technology has extended our appreciation of the normal **patterns** of **gene expression** during craniofacial and neural development, and provided the first demonstration of multiple coordinate changes in transcription patterns following teratogenic insult.

L18 ANSWER 24 OF 24 MEDLINE
AU Vaessen M J; Meijers J H; Bootsma D; Van Kessel A G
TI The cellular retinoic-acid-binding protein is expressed in tissues associated with retinoic-acid-induced malformations.
SO DEVELOPMENT, (1990 Oct) 110 (2) 371-8.
Journal code: 8701744. ISSN: 0950-1991.
AB Retinoic acid (RA) is thought to play a role in embryonic pattern formation in vertebrates. A naturally occurring gradient of endogenous RA has been demonstrated in the developing chick limb bud, while local application of RA leads to the formation of additional digits. In mammals, a well-defined spectrum of birth defects has been reported as a result of fetal exposure to excess RA. In analogy to the chick limb bud, it may be speculated that these malformations are the result of disturbance of morphogenetic RA concentration gradients. A candidate gene involved in the regulation of endogenous RA concentrations is the gene encoding cellular RA binding protein (CRABP). We have isolated a partial cDNA clone

corresponding to the chicken homolog of CRABP, and performed in situ hybridization experiments on sections of **embryos** at various stages of development. CRABP expression was detected in the CNS, the craniofacial mesenchyme, ganglia of the peripheral nervous system, the limb bud, and the visceral arch area. Our results indicate that the spatiotemporally specified **expression pattern** displayed by the CRABP **gene** exhibits a striking correspondence to the tissues that are affected by exposure of avian or mammalian **embryos** to RA. We hypothesize that CRABP plays an important role in normal embryogenesis and that embryonic tissues showing high CRABP expression are susceptible to the adverse effects of excess RA.

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